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Vitamin E suppresses telomerase activity in ovarian cancer cells

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Abstract

Background: Dietary factors influence tumor formation and progression. Vitamin E is a dietary anti-oxidant capable of eliminating free radical damage, inducing apoptosis and decreasing oncogene expression. Therefore, Vitamin E may be a strong candidate for cancer prevention and/or chemotherapeutic intervention. Since telomerase, a ribonucleoprotein uniquely expressed in over 95% of cancers, plays an important role in cellular immortalization, cell growth and tumor progression, the present study investigated the effects of Vitamin E on telomerase activity in human ovarian cancer. *Methods*: Normal and malignant ovarian surface epithelial (OSE) cells were cultured with and without D-alpha tocopheryl acetate (Vitamin E). MTS and Western immunoblot assays were used to examine the effect of Vitamin E on cell growth, survival and cytotoxicity. PCR-ELISA, RT-PCR and luciferase reporter assays were performed to determine the effect of Vitamin E on telomerase activity. *Results*: Vitamin E suppressed endogenous telomerase activity in ovarian cancer cells, but had no similar effects in telomerase-negative normal OSE cells. Vitamin E also reduced hTERT-mRNA transcript levels and reduced hTERT promoter activity maximally targeting the −976 to −578 bp promoter regions. In addition, Vitamin E improved cisplatin-mediated cytotoxicity as evidenced by reduced cancer cell growth and increased cleaved caspase 3 activity. In contrast, Vitamin E protected telomerase-negative OSE cells from cisplatin-mediated cytotoxicity as evidenced by decreased cleaved caspase 3 activity. *Conclusion*: Our data suggest that, by suppressing telomerase activity, Vitamin E may be an important protective agent against ovarian cancer cell growth as well as a potentially effective therapeutic adjuvant.

Keywords: Vitamin E acetate; Telomerase inhibition; hTERT

1. Introduction

Ovarian cancer is the leading cause of gynecologic cancer death in women in the US. It is the fourth leading cause of cancer death among women after lung, breast, and colorectal cancer [1] and is associated with a 1.7% lifetime risk [2]. It is estimated that 22,220 new cases are diagnosed in the US annually and that 16,210 women die annually from this disease [1]. The 1-year survival rate for ovarian cancer can be as high as 79% and when diagnosed in an early stage the 5-year survival rate is almost 95% [1]. However, when diagnosed at a later stage, the 5-year survival rate is generally no better than 35% [1]. Neither the survival rate nor the treatment for ovarian cancer has changed significantly for 30 years. Optimal cytoreduction followed by platinum-based chemotherapy remains the mainstay of therapy in the management of advanced epithelial ovarian cancers [3]. However, while the response rate to primary chemotherapy can be as high as 76%, response rate is dramatically reduced after relapse of the disease [4]. Platinum resistance, defined as disease recurrence less than 6 months from completion of therapy

Abbreviations: CDDP, cisplatin; FBS, fetal bovine serum; GFP, green fluorescent protein; MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenol)-2-(4-sulfophenyl)-2H-tetrazolium; OSE, ovarian surface epithelial; PCR-ELISA, polymerase chain reaction enzyme-linked immunosorbent; PMS, phenazine methosulfate; α-TEA, 2,5,7,8-tetramethyl-2*R*-(4*R*,8*R*,12-trimethyltridecyl)-chroman-6-yloxyacetic acid; TRAP, telomere repeat amplification protocol; T-TBS, tris buffered saline plus 0.1% Tween-20; Vitamin E, D-alpha tocopheryl acetate

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is an important prognostic predictor. Patients with platinum-resistant tumors have a response rate of less than 10% when retreated with platinum compounds [4]. Alternative options also have poor responses rates of 18–30% [5–7].

The majority of ovarian cancers are sporadic in origin, but about 10% of all epithelial ovarian carcinomas are associated with a hereditary predisposition and are characterized by an increased incidence and earlier onset of disease [8]. Epidemiological studies suggest that, besides race [9] and familial history of breast or ovarian cancer [8], events associated with ovarian surface epithelium (OSE) traumatization may result in aberrant OSE growth leading to ovarian epithelial carcinogenesis [10]. Specifically, it has been suggested that incessant ovulation [11] causes rapid cycles of OSE division leading to errors in DNA replication and repair resulting in the inactivation and overexpression of tumor suppressor genes and oncogenes, respectively [12]. Thus, increased age, reproductive history (nulliparity), early menarche, late menopause and fertility drug use increase the risk for ovarian cancer. In contrast, suppression of ovulation by pregnancy, lactation or oral contraceptive use decreases the risk for ovarian cancer [13]. Lifestyle factors including dietary fat intake and smoking may also increase the risk for ovarian cancer while dietary intake of Vitamins A, C, D and E may protect against ovarian cancer [13,14].

Though a number of genetic abnormalities have been identified [8], no single genetic alteration is common to all ovarian cancers. Telomerase, a ribonucleoprotein that elongates telomeric TTAGGGn DNA repeats de novo [15], is expressed in over 90% of ovarian tumors [16] and plays an important role in immortalization and carcinogenesis [17]. In the ovary, telomerase activity is absent in normal OSE and pre-malignant lesions, while tumors cells from both ascites fluid and ovarian carcinomas express telomerase activity [18]. Though telomerase regulation is complex [19,20], telomerase activity in ovarian cancer also correlates with clinical stage and tumor aggressiveness [16].

While the primary function of telomerase is the maintenance of structural integrity at the linear chromosome ends, recent studies have shown an association between telomerase activity and increased chemotherapeutic resistance consistent with poor prognosis in ovarian cancers [21]. Telomerase appears to mediate its protective effect by conferring resistance to apoptosis [22]. Likewise, we have shown that telomerase re-expression is associated with reduced caspase-mediated apoptosis and increased Bcl-2 expression in OSE cell lines [23].

While Vitamin E may protect against ovarian cancer growth and progression by reducing cancer cell growth and/or promoting apoptosis, the exact molecular mechanism(s) by which Vitamin E protects against ovarian cancer is not fully understood. Therefore, we sought to determine whether Vitamin E exerts its protective effect by targeting telomerase activity in ovarian cancer cells.

2. Materials and methods

2.1. Cell culture

The human ovarian cancer cell lines, C13, OV2008, SW 626, A2780s, A2780cp, the telomerase-negative SV-40 large T antigen transfected normal ovarian surface epithelial [7] cell lines FHIOSE 1816-575, FHIOSE 118, IOSE 80 and the telomerase-positive SV-40 large T antigen/hTERT transfected IOSE 80 + hTERT cell line [23,24] maintained in our laboratory were used in this study. Cells were maintained in Medium 199/MDCB 105 (1:1) (Sigma–Aldrich, St. Louis, MO) supplemented with 5% fetal bovine serum (FBS) (Hyclone, Logan, UT) and 10 μg/mL gentamicin (GIBCO BRL, Grand Island, NY) in a humidified 5% CO_2/95% air atmosphere. Cells were treated with 0–100 IU D-alpha tocopheryl acetate (Vitamin E) ±25 μM of cisplatin (CDDP) (Sigma–Aldrich, St. Louis, MO) for 0–72 h and then collected to perform the analyses described below.

2.2. MTS assay for cell viability

Cell growth was determined by the colorimetric 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenol)-2-(4-sulfophenyl)-2*H*-tetrazolium (MTS) assay, which is based on the soluble formazan production by dehydrogenase enzymes found in metabolically active cells (Promega, Madison, USA). Cells were trypsinized and 2×10^3 cells were seeded in 96-well plates in 0.2 mL of growth media ± Vitamin E (100 IU) ± CDDP (25 μM). Prior to collection, MTS/phenazine methosulfate (PMS) solution was added to each well and incubated at 37 °C for 1 h, at which time the absorbance at 490 nm was determined using a Dynex MRX plate reader (Dynex Technologies, Chantilly, VA) and the results expressed as mean absorbance of triplicate experiments ± S.E.

2.3. SDS-PAGE and Western blot analysis

SDS-PAGE and Western blot analysis was performed as described previously [25]. Adherent cell populations were trypsinized, pelleted for 5 min at 500 × *g*, and lysed in ice-cold lysis buffer (10 mM Tris–HCl (pH 7.5), 1 mM $MgCl_2$, 1 mM EGTA, 0.1 mM PMSF, 5 mM β-mercaptoethanol, 0.5% CHAPS, 10% glycerol) for 30 min at 4 °C. Lysates were then centrifuged at 100,000 × *g* for 1 h at 4 °C. Protein concentrations of the lysates were determined using the DC Protein Assay (Biorad, Hercules, CA) according to the manufacturer's instructions. Twenty micrograms of protein were added to 4× loading buffer (250 mM Tris pH 6.8, 8% SDS, 20% glycerol, 0.012% bromophenol blue, 4% β-mercaptoethanol), heated to 95 °C for 5 min, electrophoresed in 12.5% SDS-polyacrylamide gels, and transferred to nitrocellulose membrane (Amersham, Piscataway, NJ). All membranes were blocked for 1 h with 5% non-fat milk Tris buffered saline plus 0.1% Tween-20 (T-TBS) and incubated